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Research Report

Constitutive androstane receptor upregulates Abcb1 and Abcg2 at the blood–brain barrier after CITCO activation

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ABSTRACT

ATP-driven efflux transporters are considered to be the major hurdle in the treatment of central nervous system (CNS) diseases. Abcb1 (P-glycoprotein) and Abcg2 (breast cancer resistance protein/brain multidrug resistance protein) belong to the best known ABC-transporters. These ABC-transporters limit the permeability of the blood–brain barrier and protect the brain against toxic compounds in the blood but on the other hand they also reduce the efficacy of CNS pharmacotherapy. Even after 40 years of extensive research, the regulatory mechanisms of these efflux transporters are still not completely understood. To unravel the efflux transporter regulation, we analyzed the effect of the nuclear receptor CAR (constitutive androstane receptor) on the expression of Abcb1 and Abcg2 in primary cultures of porcine brain capillary endothelial cells (PBCEC). CAR is a xenobiotic-activated transcription factor, which is, like the other important nuclear receptor pregnane X receptor (PXR), highly expressed in barrier tissue and known to be a positive regulator of ABC-transporters. We demonstrate that activation of porcine CAR by the human CAR (hCAR) ligand CITCO (6-(4-chlorophenyl)-imidazo[2,1-b]thiazole-5-carbaldehyde) leads to an up-regulation of both transporters, whereas the mouse-specific CAR ligand TCPOBOP (1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene) had no effect on transporter expression. The stimulation of PBCEC with CITCO caused a significant up-regulation of both efflux-transporters on RNA-level, protein level and transport level. Furthermore the additional application of a CAR inhibitor significantly decreased the transporter expression to control niveau. In conclusion our data prove CAR activation only by the human ligand CITCO leading to an increased ABC-transporter expression and transport activity.

Abbreviations: ABC-transporter, ATP-binding cassette transporter; BBB, blood–brain barrier; BCRP, breast cancer resistance protein; BMDP, brain multidrug resistance protein; CAR, constitutive androstane receptor; CYP, cytochrome P450; pgCAR, porcine CAR; CITCO, 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazol-5-carbaldehyd-O-(3,4-dichlorobenzyl)oxim; CNS, central nervous system; FTC, Fumitremorgin C; hCAR, human CAR; MDR, multidrug resistance; mCAR, murine CAR; Mrp2, multidrug resistance-associated protein 2; PBCEC, porcine brain capillary endothelial cells; PCR, polymerase chain reaction; pgCAR, porcine CAR; PSC833, 6-[(2S,4R,6E)-4-methyl-2-(methylamino)-3-oxo-6-octenoic acid]-7-L-valine-cyclosporin A; PXR, pregnane X receptor; RXR, retinoid X receptor; TCPOBOP, 1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene; TEER, transendothelial electrical resistance

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1. Introduction

Drug delivery to the central nervous system (CNS) is one of the toughest hurdles of pharmacotherapy. To a large extent this is due to the inability of many drugs to cross the blood–brain barrier and reach their site of action in sufficiently high concentrations (Miller, 2010). The structural basis of the blood–brain barrier (BBB) within the capillary vessels consists of endothelial cells which are connected and sealed by tight junctions (Wolburg and Lippoldt, 2002). The blood–brain barrier phenotype requires the expression of ATP-driven efflux transporters to protect the healthy brain but these transporters also impede pharmacotherapy of CNS disorders. ABC-transporter play a prevalent role in controlling the passage of xenobiotics and toxins to the brain (Pardridge, 2010) but still their regulatory mechanisms are not completely understood.

Two most prominent ABC-transporters of the BBB are Abcg2 (BMDP, brain multidrug resistance protein; BCRP, breast cancer resistance protein) (Eisenblätter et al., 2003) and Abcb1 (P-glycoprotein) which are expressed at the luminal plasma membrane (Abbott et al., 2010). They recognize a wide range of drugs as substrates, like anti-epileptics, anti-depressants or anticancer drugs and constitute a selective and active transport barrier (Begley and Brightman, 2003). Abcb1 and Abcg2 are of particular importance for studying the phenomenon of the multidrug resistance (MDR) because they exhibit functional activity at the human blood–brain barrier (Dauchy et al., 2009). It is known that increased expression of Abcb1 selectively tightens the blood–brain barrier to drugs that are potential ABC-transporter substrates (Miller et al., 2008). Experiments with Abcb1-knockout mice revealed increased brain drug concentration (10- to 100-fold) in comparison to wildtypes (Schinkel et al., 1994). Another study, in which Abcg2 was blocked by specific inhibitors evidently demonstrated an increased mitoxantrone brain accumulation in Abcb1-knockout animals compared to wildtype. The same study also revealed an increased Abcg2 gene expression in Abcb1(-/-)-mice supporting the idea of compensatory gene expression of both transporters (Cisternino et al., 2004).

One potential regulator of ABC-transporters is the constitutive androstane receptor (CAR). CAR belongs to the superfamily of nuclear receptors and is highly expressed in the liver and the epithelial cells of the small intestinal villi. Recent studies show that CAR is also expressed in brain capillary endothelial cells of mouse, rat, pig and human (Bauer et al., 2004; Dauchy et al., 2008; Nannelli et al., 2010; Ott et al., 2009). CAR, as well as PXR (pregnane X receptor) coordinately upregulate the expression of phase I and phase II enzymes like cytochrome P450 (CYP) and increase the excretory transport mediated by ABC-transporters (Wang and Negishi, 2003; Xu et al., 2005). Usually CAR is cytoplasmic in the naive state and translocate to the nucleus upon activation by potential ligands (Maglich et al., 2003; Moore et al., 2000). Unlike other nuclear receptors CAR does not necessarily require the direct binding of ligands. Bilirubin has been shown to activate the transcription factor CAR without interacting with it directly (Moore et al., 2000; Swales and Negishi, 2004). Recently Wang et al. (2010) have identified CAR as a positive regulator of Abcb1, Abcg2 and Mrp2 (multidrug resistance protein 2) expression in mouse capillaries (Wang et al., 2010). When these capillaries were exposed to the mouse-specific CAR-ligand TCPOBOP (1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene) transporter expression and activity was increased. Similar studies using human cerebral microvessel endothelial cells revealed increased mRNA expression of Abcb1 after treatment with CITCO (6(4-chlorophenyl)imidazo[2,1-b][1,3]thiazol-5-carbaldehyd-O-(3,4-dichlorobenzyl)oxim), a specific human (hCAR) ligand (Chan et al., 2011). Interestingly TCPOBOP is a potent activator of mCAR but lacks any activity on hCAR (di Masi et al., 2009).

The porcine constitutive androstane receptor (pgCAR) exhibits a 83.6% amino acid sequence identity to hCAR whereas mouse CAR (mCAR) shows just 72.6% homology (di Masi et al., 2009). Also Gray et al. 2009 confirmed these findings by showing that the ligand binding domain (LBD) of pgCAR was 84% homologous to the LBD of hCAR, the mCAR domain revealed just 71% homology at the amino acid level (Gray et al., 2009). Due to the lower degree of sequence homologies to hCAR, rodent models are unsuitable to predict xenobiotic interaction of hCAR. The PBCEC represent a more suitable model to contribute to a better understanding of ABC-transporter regulation by nuclear receptors.

In the present study our aim was to analyze the influence of the orphan receptor CAR on the expression of Abcb1 and Abcg2. A porcine blood–brain barrier cell culture model was used for all experiments. Activation of CAR was achieved by stimulation with the specific mCAR ligand TCPOBOP and the hCAR ligand CITCO. Our results indicate functional activity of CAR in porcine brain capillary endothelial cells after incubation with CITCO but not with TCPOBOP. CITCO stimulation triggered an up-regulation of both ABC-transporters on RNA, protein and transport level.

2. Results

2.1. *pgCAR expression and activation in PBCEC*

To analyze the regulatory influence of CAR on the ABC-transporters a verification of CAR expression in porcine brain capillary endothelial cells was required. By means of immuno-staining nostaining analysis CAR expression was detected in 7 days old PBCEC (Fig. 1). CAR is a transcription factor which is inactive in the cytoplasm and translocates to the nucleus upon activation. Although CAR is a ligand activated nuclear receptor, direct binding of the ligand is not necessarily required. Nevertheless, in this study the only known specific ligands for mCAR (TCPOBOP) and hCAR (CITCO) were used. Stimulating the PBCEC with 1 μM CITCO for 24 h revealed an increased CAR immunofluorescence (green) in the perinuclear region as well as in the nucleus in comparison to untreated control cells. In contrast to this, stimulation of PBCEC with TCPOBOP did not show any change in fluorescent signal of CAR.

2.2. *Influence of pgCAR activation on the TEER of PBCEC*

The TEER is not only a method to measure the integrity of the cell monolayer but also a sensitive indicator for cell viability.